Media Recipes for Sweet Potato

MS multiplication medium (solid) - 1000 ml

✓ To a small volume of double distilled water (ddH_2O) add:

MS basal medium w/vitamins¹ 4.43 g (prepackaged as M519²)

Casein hydrolysate 1.0 g Sucrose 30.0 g Sequestrene®138 0.1 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Add:

Phytagel™^{3*}

2.0 g

- ✓ Mix and heat until boiling
- ✓ Dispense into stacked Magenta®⁴ GA7* culture vessels (75 ml/vessel)
- ✓ Autoclave

MS+BA nodal medium (solid) - 1000 ml

 \checkmark To a small volume of double distilled water (ddH₂0) add:

MS basal medium w/vitamins¹ 4.43 g (prepackaged as M519²)
BA (6-benzylaminopurine) 0.5 mg
Casein hydrolysate 1.0 g
Sucrose 30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Add:

Phytagel $^{\text{Im}3}$ * 2.0 g

- ✓ Mix and heat until boiling
- ✓ Autoclave
- ✓ In laminar flow hood, dispense slightly cooled liquid into sterile 100x25 mm Petri dishes. Allow to cool completely and then wrap dishes with Parafilm®* until used.

MS preincubation medium (liquid) - 1000 ml

✓ To a small volume of double distilled water (ddH₂0) add:

MS basal medium w/vitamins¹ 4.43 g (prepackaged as M519²)
Casein hydrolysate 1.0 g
Sucrose 30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Heat and stir until well blended
- ✓ Dispense into desired vessels
- ✓ Autoclave

Ca-free MS+3 % (w/v) Na-alginate medium (liquid) - 100 ml

✓ To a small volume of double distilled water (dd H_2 0) add:

Potassium nitrate (KNO ₃)	1.9 g
Ammonium nitrate (NH ₄ KNO ₃)	1.65 g
Magnesium sulfate (MgSO ₄)	0.1807 g
Potassium phosphate, monobasic (KH ₂ PO ₄)	0.17 g
Iron stock ⁵	1.0 ml
MS micronutrients ^{1,5}	1.0 ml
MS vitamins ^{1,5}	1.0 ml
Reagent grade sucrose	25.67 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (100 ml) with ddH₂0
- ✓ Adjust pH to 5.8
- ✓ Add:

Alginic acid sodium salt, 2% viscosity (Sigma^{®4} A2158*) 3.0 g

To prevent clumping, add the alginic acid slowly to rapidly stirring medium. A homogenizer with a propeller-type stirring blade works well for this step.

- ✓ Continue stirring until well blended and alginic acid is completely dissolved (~ 20 minutes)
- ✓ Dispense into desired vessels
- ✓ Autoclave

Iron stock solution (100x) (liquid) - 500 ml

✓ To a small volume of double distilled water (ddH₂0) add:

NaEDTA, disodium salt, dihydrate (NA₂EDTA_{*}2H₂0) 1.86 g

- ✓ Stir until NaEDTA is completely dissolved
- ✓ In a separate vessel containing a small volume of ddH₂0 add:

Ferric sulfate (FeSO_{4*}7H₂O)

1.39 g

- ✓ Heat and stir until the ferric sulfate is completely dissolved. Allow solution to cool completely
- ✓ Combine the NaEDTA solution with the ferric sulfate solution
- ✓ Bring to volume (500 ml) and stir until the solution color turns golden
- ✓ Dispense into an amber vessel to prevent photodegredation. Store at 2-4°C

MS¹ micronutrient stock solution (100x) (liquid) – 500 ml

✓ To a small volume of double distilled water (ddH_2O) add:

Boric acid (H ₃ BO ₃)	0.31 g
Cobalt chloride (CoCl ₂ * 6H ₂ 0)	0.00125 g
Cupric sulfate (CuSO _{4*} 5H ₂ 0)	0.00125 g
Zinc sulfate (ZnSO _{4*} 7H ₂ 0)	0.43 g
Molybdic acid, sodium salt, dihydrate (NaMoO _{4*} 2H ₂ O)	0.0125 g
Manganese sulfate (MnSO _{4*} H ₂ 0)	0.845 g
Potassium iodide (KI)	0.0415 g

- ✓ Heat and stir until boiling and dry ingredients have completely dissolved.
- ✓ Bring to final volume (500 ml) with ddH₂0
- ✓ Dispense into desired vessel and store at 2-4°C or aliquot and store in freezer

MS¹ vitamin stock solution (100x) (liquid) – 500 ml

✓ To a small volume of double distilled water (ddH₂0) add:

Glycine (free base)	0.1 g
Myo-inositol	5.0 g
Nicotinic acid (free base)	0.025 g
Pyridoxine HCl	0.025 g
Thiamine HCl	0.005 g

- ✓ Stir until ingredients are well blended
- ✓ Bring to final volume (500 ml) with ddH20

Dispense into desired vessel and store at 2-4°C or aliquot and store in freezer

0.1 M calcium chloride+MS encapsulation medium (liquid) – 1000 ml

✓ To a small volume of double distilled water (ddH_2O) add:

MS basal medium w/vitamins¹ 4.43 g (prepackaged as M519²)
Calcium chloride, dihydrate (CaCl_{2*}2H₂0) 14.7 g
Casein hydrolysate 1.0 g
Sucrose 30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Heat and stir until well blended
- ✓ Dispense into desired vessels
- ✓ Autoclave

0.3 M sucrose+MS preculture medium (liquid) – 1000 ml

✓ To a small volume of double distilled water (ddH₂0) add:

MS basal medium w/vitamins¹ 4.43 g (prepackaged as M519²)
Casein hydrolysate 1.0 g
Reagent grade sucrose 02.7 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Heat and stir until well blended
- ✓ Dispense into desired vessels
- ✓ Autoclave

1.6 M sucrose+2 M glycerol+MS loading medium (liquid) - 500 ml

✓ To a small volume of double distilled water (ddH₂0) add:

MS basal medium w/vitamins¹ 2.21 g (prepackaged as M519²)
Casein hydrolysate 0.5 g
Reagent grade sucrose 273.85 g
Glycerol 115.0 g (w/v)

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (500 ml) with ddH₂0
- ✓ Adjust pH to 5.8
- ✓ Heat and stir until well blended
- ✓ Dispense into desired vessels
- ✓ Autoclave

PVS2+1/2 strength MS (liquid) – 250 ml

✓ Combine:

Glycerol 75.0 g (w/v)
Ethylene glycol 33.8 ml

DMSO (dimethylsulfoxide) 34.1 ml

Reagent grade sucrose 34.25 g

MS basal medium w/vitamins¹ 0.554 g (prepackaged as M519²)

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (250 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Filter sterilize
- ✓ Dispense into an amber vessel to prevent photodegredation

1.2 M sucrose+MS minus NH₄ medium (liquid) – 500 ml

✓ To a small volume of double distilled water (ddH₂0) add:

MS modified basal salt mixture w/o ammonium nitrate (prepackaged as M571²) 1.34 g
Reagent grade sucrose 5.0 g
Casein hydrolysate 0.5 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (500 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Heat and stir until well blended
- ✓ Dispense into desired vessels
- ✓ Autoclave

Ipomoea recovery I medium (solid) – 1000 ml

✓ To a small volume of double distilled water (ddH_20) add:

MS modified basal salt mixture w/o ammonium nitrate (prepackaged as M571 2) 2.68 g Casein hydrolysate 1.0 g GA $_3$ (gibberellic acid) 1.0 mg BA (6-benzylaminopurine) 0.5 mg Sucrose 30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Add:

Phytagel™^{4*}

2.0 g

- ✓ Heat and stir until boiling
- ✓ Autoclave
- ✓ In laminar flow hood, dispense hot liquid into sterile 60x15 mm Petri dishes. Allow to cool completely and then wrap dishes with Parafilm® until used.

Ipomoea recovery II medium (solid) - 1000 ml

✓ To a small volume of double distilled water (ddH₂0) add:

MS basal medium w/vitamins¹ 4.43 g (prepackaged as M519²) Casein hydrolysate 1.0 g

GA₃ (gibberellic acid) 0.5 mg Sucrose 30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂0
- ✓ Adjust pH to 5.7
- ✓ Add:

Phytagel™⁴*

2.0 g

- ✓ Heat and stir until boiling
- ✓ Autoclave
- ✓ In laminar flow hood, dispense hot liquid into sterile 100x25 mm Petri dishes. Allow to cool completely and then wrap dishes with Parafilm®* until used.

¹Murashige & Skoog, 1962

²Phytotechnology Laboratories, Shawnee Mission, KS*

³Sigma-Aldrich, St. Louis, MO*

⁴Magenta Corp. Chicago, IL*

⁵Recipe follows

*Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.